

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.	:	10/478,262	Confirmation No. 2549
Applicants	:	Evert J. BUNSCHOTEN et al.	
Filed	:	May 25, 2004	
Title	:	PHARMACEUTICAL COMPOSTION FOR USE IN HORMON REPLACEMENT THERAPY	
Group Art Unit	:	1617	
Examiner	:	San Ming R. HUI	
Customer No.	:	28289	
Application No.	:	10/478,264	Confirmation No. 4962
Applicants	:	Evert J. BUNSCHOTEN et al.	
Filed	:	May 25, 2004	
Title	:	USE OF ESTROGEN COMPOUNDS TO INCREASE LIBIDO IN WOMEN	
Group Art Unit	:	1617	
Examiner	:	San Ming R. HUI	
Customer No.	:	28289	
Application No.	:	10/478,357	Confirmation No. 3771
Applicants	:	Evert J. BUNSCHOTEN et al.	
Filed	:	May 25, 2004	
Title	:	DRUG DELIVERY SYSTEM COMPRISING A A TETRAHYDROXYLATED ESTROGEN FOR USE IN HORMONAL CONTRACEPTION	
Group Art Unit	:	1617	
Examiner	:	San Ming R. HUI	
Customer No.	:	28289	

Application No.	:	10/517,509	Confirmation No. 1291
Applicants	:	Herman J. T. Coelingh Bennink et al.	
Filed	:	June 13, 2005	
Title	:	METHOD OF TREATING HUMAN SKIN AND	
	:	A SKIN CARE COMPOSITION FOR USE IN	
	:	SUCH METHOD	
Group Art Unit	:	1617	
Examiner	:	Samira JEAN-LOUIS	
Customer No.	:	28289	

### **DECLARATION**

I, Speroff, Leon declare and state the following:

1. A detailed listing of my publications, together with details of my education, are given in my *curriculum vitae* which is attached as Exhibit A.

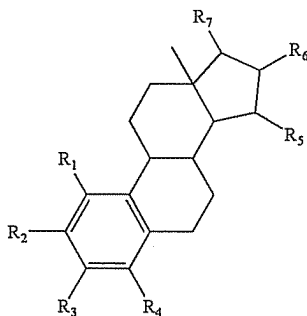
2. Based on my academic training and professional experience, I consider myself an expert in the field of estrogen-related therapies and treatments, and I was such a person in 2001 and 2002.

3. I have received copies of patent applications that I understand were filed in the United States and correspond to the above-captioned applications.

4. I understand that the above mentioned patent applications relate to:

- new methods of contraception (Appln. 10/478,357);
- new methods of hormone replacement therapy (Appln. No. 10/478,262);
- a new method of increasing female libido (Appln. No. 10/478,264);
- a new method treating vaginal dryness (Appln. No. 10/517,509).

The aforementioned methods have in common that they comprise administration of the following estrogenic component:



wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> independently are a hydrogen atom, a hydroxyl group or an alkoxy group with 1-5 carbon atoms. R<sub>5</sub>, R<sub>6</sub> and R<sub>7</sub> are hydroxyl groups. No more than three of R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are hydrogen atoms. The invention also includes using variations of this formula, such as precursors capable of liberating a substance according to the aforementioned formula and mixtures of one or more of the aforementioned substances and/or precursors. One embodiment of the aforementioned formula is estetrol.

5. I have also received copies of Office Actions that have been issued in relation to the above referenced pending patent applications. Specifically, I have received copies of the following Office Actions:

OA-1 - 10/478,262 (Non-final Office Action mailed on May 15, 2008)

OA-2 - 10/478,264 (Non-final Office Action mailed on March 6, 2008)

OA-3 - 10/478,357 (Non-final Office Action mailed on May 16, 2008)

OA-4 - 10/517,509 (Non-final Office Action mailed on March 26, 2008)

6. I have further received copies of the following references that I have been told have been cited in the above mentioned Office Actions against the independent claims of the above referenced pending patent applications.

Publications mentioning estetrol:

- D1 US 5,211,952 (Spicer et al.) – cited in OA-4
- D2 US 5,340,584 (Spicer et al.) – cited in OA-2
- D3 US 5,340,586 (Pike et al.) – cited in OA-1, OA-2 and OA-3
- D4 US 2004/0192598 (Kragie) – cited in OA-4
- D5 Holinka, Biology of Reproduction, 1979; 20(2): 242-246 <sup>1</sup> – cited in OA-1 and OA-2
- D6 Holinka, Biology of Reproduction, 1980; 20(4): 913-926 <sup>2</sup> – cited in OA-1 and OA-2

Publications not mentioning estetrol:

- E1 Ullom –Minnich, American Family Physician, 1999; 60: 194-202 – cited in OA-1
- E2 Katzung, Basic and Clinical Pharmacology, 6<sup>th</sup> ed., 1995, 608-624 – cited in OA-3
- E3 Willhite et al. (Pharmacotherapy, 2001, vol. 21, issue 4, 464-480 – cited in OA-4
- E4 Sitruk-Ware et al. (Schweiz. Rundsch., Med. Praxis, 1997, vol. 86, No. 33, 1245-1248 – cited in OA-4

It is my understanding that the independent claims of the pending patent applications that are the subject of this Declaration were rejected as being unpatentable over the above cited references under 35 U.S.C. 103(a) (obviousness). I have been asked to comment on my understanding of the state of the art prior to June 11, 2002, which I understand is the priority date for Appln. No. 10/517,509. Particularly, I have been asked whether, prior to June 11, 2002, a person of ordinary skill in the art would have considered it obvious to use estetrol in the pharmacological applications listed in § 4. More particularly, I have been asked whether, prior to the June 11, 2002, a person of ordinary skill in the art would have been motivated to use estetrol in the pharmacological applications listed in § 4, and whether the discovery that estetrol was pharmacologically useful in these applications is unexpected and surprising.

7. It is my view that, prior to June 11, 2002, for the reasons presented below, a person of ordinary skill in the art would not have expected estetrol to be pharmacologically useable, and that the Applicants were the first to discover the pharmacological usefulness of estetrol. In addition, and more particularly, it is my opinion that, prior to June 11, 2002, a person of ordinary skill in the art would not have expected estetrol to be pharmacologically active when orally administered.

8. I declare that before June 11, 2002 I had no knowledge of any concrete pharmacological application of estetrol. Furthermore, before June 11, 2002, I did not expect that estetrol can be used effectively as a drug in therapeutic treatments or in hormonal contraceptives. Based on the data from scientific literature that was available before June 11, 2002, I would have expected estrogenic activity of estetrol to be too low for pharmacological applications, such as the ones recited in Applicants' claims.

9. My view that a person of ordinary skill in the art would not have expected estetrol to be pharmacologically active is supported by leading textbooks in the field of endocrinology. In "Clinical Gynecologic Endocrinology and Infertility" <sup>3</sup> estetrol is solely mentioned in Chapter

8 (The Endocrinology of Pregnancy) under the subheading "Measurement of Estrogen in Pregnancy" (page 287) and in the index. On page 287 it is stated that "Estetrol (15alpha-hydroxyestriol) is formed from a fetal precursor and is very dependent on 15alpha-hydroxylation activity in the fetal liver. The capacity for 15alpha-hydroxylation of estrogens increases during fetal life, reaching maximum at term. This activity then declines during infancy and is low, absent or undetectable in adults. There is no clinical use for maternal blood or urine estetrol measurements during pregnancy. The clinical use of maternal blood and urine estetrol measurements is of no advantage over the usual estriol assessment."

10. The unexpected pharmacological activity of estetrol is associated with Applicants' discovery that estetrol has a surprisingly long *in vivo* elimination half-life. Applicants' finding that estetrol has a terminal elimination half-life of about 28 hours, which is very much longer than that of the other pregnancy hormone estriol (5-10 minutes), was very unexpected and provided the clue towards its pharmacological usefulness as will be further explained below.

11. It is my understanding that the claims of the pending patent applications that are the subject of this Declaration were rejected as obvious because it has been asserted by USPTO examiners that it is known from the references cited in § 6:

- (i) to use estrogens with or without progestins in HRT (reference E1);
- (ii) to use a combination of estrogen and progestin in hormonal contraceptives (reference E2);
- (iii) to use estrogen to treat decreased libido in women taking GnRH agonists (reference D2); and to use a combination of estrogen and androgen to treat decreased libido in oophorectomized women (reference D3);
- (iv) to treat vaginal dryness by administering estrogen (references D1, D4, E3, E4).

12. Assuming that the references cited by the USPTO examiners disclose the information contained in § 11 (i) to (iv), I do not think that, in view of these references, it would have been obvious to use estetrol in pharmacological applications described in § 4. I appreciate that the cited references D1 to D4 contain references to estetrol within a lengthy list of other estrogens. Furthermore, I have read the cited papers published by Holinka et al ("Holinka articles")<sup>1,2</sup>, which report that parenterally administered estetrol produced estrogenic changes in the immature rat uterus.

13. I declare that although the cited references D1 to D4 list estetrol among candidate estrogens for pharmaceutical use, it is my view that a person of ordinary skill in the art having knowledge of the aforementioned patent publications D1 to D4 and the "Holinka articles", would not have expected estetrol to be pharmacologically useable for the reasons presented below.

14. The mere mentioning of estetrol in a long list of candidate estrogens in D1 to D4 without any experimental data to support the viability of pharmaceutical uses described in these patents, in my view would not have provided a person of ordinary skill in the art with any motivation to actually employ estetrol in these pharmaceutical uses. Furthermore, the aforementioned US patent publications would not have provided a person of ordinary skill in the art with any motivation to replace the estrogens employed in the uses (i) to (iv) mentioned in §11 with estetrol.

15. In Holinka (1979)<sup>1</sup> the estrogenic activity of estetrol was evaluated by examination of uterine responses to subcutaneous administration of estetrol in doses of 10 and 50 µg/100g body mass. The effects were compared to those obtained by administration of 1 µg/100g body mass estradiol or estriol. The last paragraph of the abstract of Holinka (1979) reads as follows "It is concluded that estetrol administered as a single dose or in 2 doses at a 24 h interval is a weak estrogen which produces effects of short duration. It cannot, however, be considered entirely devoid of estrogenic activity, even though true uterine hyperplasia, as estimated by DNA content, was not promoted by administration of the two 50 µg/100 g bw doses of estetrol".

16. Holinka (1980)<sup>2</sup> describes the results of a study that aimed to extend the study described in Holinka (1979). In this follow-up study estrogenic effects on immature rat uterus of estetrol and the antiestrogen tamoxifen were compared with those of estradiol and estriol. This time, estetrol was injected subcutaneously for 3 days at a dose of 50 µg/100g body mass, a dose 50 times greater than the dosages of estradiol and estriol that were administered subcutaneously (at a dose of 1 µg/100g body mass). The last paragraph of the abstract of Holinka (1980) reads as follows: "In general estradiol treatment promoted the most marked changes, followed by tamoxifen, estriol and estetrol. On the basis of the present biochemical and morphological results, it is concluded that estetrol and tamoxifen have estrogenic effects on the immature rat uterus. However, the estrogenic potency of estetrol, relative to estradiol or estriol was low at the dosage and timing of administration used in these experiments; effects of estetrol introduced in the circulation at a constant rate were not evaluated. These results suggest that the conversion of estradiol to estetrol in the human fetus represent an efficient mechanism of inactivation of the placental hormone." Specifically, even though Holinka et al administered 50 times more estetrol than estradiol or estriol, the observed uterotrophic effects of estetrol were still smaller than those of estradiol or estriol. Thus, from Holinka (1980), one of ordinary skill in the art would expect estetrol to be more than 50 times less effective than a weak estrogen, such as estriol.

17. It is my view that a person of ordinary skill in the art would have deduced from the Holinka articles that estetrol has estrogenic activity, but that it is a much weaker estrogen than the already weak estrogen estriol, given that estetrol injected subcutaneous at 50 µg/100g body mass exhibited less estrogenic activity than estriol injected subcutaneous at 1 µg/100g body mass. Estriol is a very weak estrogen due to its low receptor affinity in combination with its very short half-life of 5-10 minutes. Since the Holinka articles teach that estrogenic activity of estetrol is at least 50 times lower than that of a weak estrogen for which very few practical applications exists, the Holinka articles would not have provided a motivation for a person of ordinary skill in the art to investigate the potential pharmacological usefulness of estetrol.

18. Applicants have demonstrated that, contrary to what a person of ordinary skill in the art would have expected, estetrol is pharmacologically very active. The unexpected pharmacological activity of estetrol is associated with its surprisingly long *in vivo* elimination half-life. Whereas, under comparable conditions, the human estrogens estradiol and estriol have terminal elimination half-lives of about 30 minutes and 5-10 minutes respectively, estetrol has a terminal elimination half-life of about 28 hours. A person of ordinary skill in the art would have expected estetrol to be more comparable to estriol than estradiol given that (i) estetrol differs from estriol by only 1 hydroxy group and from estradiol by 2 hydroxy groups and (ii) both estriol and estetrol are produced during pregnancy. Hence, Applicants' finding that estetrol has a terminal elimination half-life that is 168-336 higher than that of the other pregnancy hormone

estriol, was very unexpected and provided the clue towards its pharmacological usefulness. Based on my knowledge of the relevant art, I conclude that Applicants are the first to have discovered estetrol's pharmacological usefulness. As explained herein before, it is my view that, prior to June 11, 2002, a person of ordinary skill in the art would not have anticipated this usefulness.

19. In addition, I conclude that Applicants are the first to have discovered estetrol's high oral bioavailability. This finding is truly surprising as other human estrogens, notably estradiol, estriol and estrone, exhibit low oral bioavailability because they are largely metabolized into inactive metabolites during the so called "first pass" through the liver after oral administration. It is my opinion that, given that estetrol's estrogen receptor affinity was known to be considerably lower than that of estradiol and estriol, a person of ordinary skill in the art, being aware that known human estrogens are largely metabolized during the first pass, could not have anticipated the high oral bioavailability of estetrol. Thus, in my view, prior to June 11, 2002, a person of ordinary skill in the art could not have anticipated estetrol's oral pharmacological activity.

20. As mentioned herein before, it is my view that a person of ordinary skill in the art could not have anticipated the advantageous pharmacological properties of estetrol that Applicants have described in the above referenced pending patent applications and that have been reported in scientific articles that were published after June 11, 2002. In particular, such a skilled person could not have foreseen the favorable pharmacokinetic (ADME) and pharmacodynamic properties of estetrol. These favorable properties of estetrol are remarkable since they are much less manifest in other human estrogens, notably estradiol, estriol and estrone. The unexpected favorable properties of estetrol that have been described by Applicants in the aforementioned pending patent applications and that were not known before June 11, 2003 include:

A. Long *in vivo* elimination half-life in the human

- In the first human study with estetrol, a dose-independent terminal elimination half-life of about 28 hours after single oral administration to early postmenopausal women was demonstrated <sup>4,5</sup>. Terminal elimination half-lives of the human estrogens estradiol and estriol under comparable conditions are about 30 minutes and 5-10 minutes respectively <sup>3</sup>.

B. No binding affinity for sex hormone binding globulin (SHBG)

- Competitive ligand binding assays did not detect any binding of estetrol to the SHBG steroid-binding sites <sup>4,6</sup>. By contrast, estradiol is bound by SHBG with high affinity <sup>6</sup>.

C. No ER $\alpha$ -mediated increase in SHBG production by HepG2 or Hep89 cells

- Fluorometric assays in wild-type human HepG2 and Hep89 cells showed that estetrol does not stimulate ER $\alpha$ -mediated increases in SHBG production by these cells, in contrast to estradiol and estriol <sup>4,6</sup>.

D. No conversion to other active metabolites

- Estetrol is an end-stage product of estrogen metabolism <sup>4,5,7</sup>. In contrast, especially after oral administration, estradiol is rapidly and reversibly converted by the liver to the estrogenic metabolites estrone and estrone sulfate.


- E. No significant inhibition of P450 enzymes
- At a concentration of 10  $\mu\text{mol/l}$  estetrol has no inhibitory effect on any recombinant human P450 enzymes CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. In contrast, at the same concentration estradiol moderately inhibits CYP1A2 and strongly inhibited CYP2C19<sup>4,7</sup>.
- F. Highly selective binding to estrogen receptors ER $\alpha$  and ER $\beta$
- Estetrol tested at a prime concentration of 10  $\mu\text{mol/l}$ , did not show significant (>20%) inhibition of the binding of the respective ligands in 123 of the 124 assays studied (Estetrol only inhibited binding of prazosin at the adrenergic  $\alpha_{1B}$  receptor by 23%)<sup>4,7</sup>.
- G. Estrogen agonist in bone, vagina, myometrium, endometrium and brain, but estrogen antagonist in breast tumor tissue in the presence of estradiol
- Estetrol significantly and dose-dependently inhibited the OVX-related increase in osteocalcin levels, increased bone mineral density and content, and increased bone strength<sup>4,8</sup>.
  - Estetrol is effective in preventing temperature rises dose-dependently in an animal model considered representative for menopausal vasomotor symptoms<sup>4,9</sup>.
  - In the modified Allen-Doisy test estetrol was found to have dose-dependent estrogenic effects on the vagina and on the uterus of ovariectomized rats including the endometrium<sup>4,10</sup>.
  - Estetrol at a twice-daily dose of 0.3 mg/kg and above effectively inhibited ovulation in regularly cycling female rats<sup>4,11</sup>.
  - Estetrol dose-dependently prevents the growth of chemically induced (DMBA) mammary tumors in rats and has the potential to reduce the number and size of pre-existing mammary tumors<sup>4,12</sup>. By contrast it is well-established that estradiol has proliferative effects on breast tumor cells and tissue.
- H. Oral absorption in the human with a strong dose-response relationship suggesting high oral bioavailability
- In a first-in-human study four single doses of 0.1, 1, 10 and 100 mg estetrol were administered orally to early postmenopausal women. High oral bioavailability, a strong dose-response relationship and a long elimination half-life (see A) were found. For the first time (oral) pharmacodynamic effects of estetrol were observed since the data showed a strong suppression of follicle stimulating hormone (FSH) with the 100 mg dose and a dose-dependent inhibition of luteinizing hormone (LH) levels<sup>4,5</sup>.

The above mentioned features A to D imply that the estrogenic activity of estetrol is much more pronounced than could have been anticipated on the basis of the estrogen receptor affinity studies described in scientific literature before June 11, 2002. Features E and F indicate that it is unlikely that estetrol administration will induce undesirable side-effects. Feature G indicates that estetrol may suitably be used as a drug in estrogen or hormone replacement therapy (ERT/HRT) including the prevention of osteoporosis (US 10/478,262), the treatment of female sexual dysfunction (US 10/478,264), topical treatment of vaginal atrophy (US 10/517,509) and as the estrogenic component in contraceptives (US 10/478,357). Feature H indicates that estetrol has potential as a once-a-day oral drug for human use.



21. I have not been compensated for the execution of this declaration, or any time I spent relating to this declaration.

22. I declare further that all statements made herein are true to my knowledge; and that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

  
Speroff, Leon

8-26-08  
Date

## References

- <sup>1</sup> Holinka et al., *In vivo effects of estetrol on the immature rat uterus*. Biol Reprod 20 (1979) 242-6.
- <sup>2</sup> Holinka et al., *Comparison of effects of estetrol and tamoxifen with those of estriol and estradiol on the immature rat uterus*. Biol Reprod 22 (1980) 913-26.
- <sup>3</sup> Leon Speroff, Robert H. Glass and Nathan G. Kase. *Clinical Gynecologic Endocrinology and Infertility*. Baltimore, Maryland, USA. Lippincott Williams & Wilkins, 1999.
- <sup>4</sup> Coelingh Bennink et al., *Estetrol Review: profile and potential clinical applications*, Climacteric 2008; 11 (Suppl 1): 47-58
- <sup>5</sup> Visser et al., *First human exposure to exogenous single-dose oral estetrol in early postmenopausal women*, Climacteric 2008; 11 (Suppl 1): 31-40
- <sup>6</sup> Hammond et al., *Estetrol does not bind sex hormone binding globulin or increase its production by human HepG2 cells*, Climacteric 2008; 11 (Suppl 1): 41-46
- <sup>7</sup> Visser et al., *In vitro effects of estetrol on receptor binding, drug targets and human liver cell metabolism*, Climacteric 2008; 11 (Suppl 1): 64-68
- <sup>8</sup> Coelingh Bennink et al., *Oral bioavailability and bone-sparing effects of estetrol in an osteoporosis model*, Climacteric 2008; 11 (Suppl 1): 2-14
- <sup>9</sup> Holinka et al., *Preventive effect of oral estetrol in a menopausal hot flush model*, Climacteric 2008; 11 (Suppl 1): 15-21
- <sup>10</sup> Heegaard et al., *Estrogenic uterovaginal effects of oral estetrol in the modified Allen-Doisy test*, Climacteric 2008; 11 (Suppl 1): 22-28
- <sup>11</sup> Coelingh Bennink et al., *Ovulation inhibition by estetrol in an in vivo model*, Contraception 2008; 77: 186-190
- <sup>12</sup> Coelingh Bennink et al., *Estetrol, a pregnancy specific human steroid, prevents and suppresses mammary tumor growth in a rat model*, Climacteric 2008; 11 (Suppl 1): 29